

# Positioning nuclei within the cytoplasm of striated muscle fiber

## Cooperation between microtubules and KASH proteins

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**S**triated muscles contain a tightly ordered cytoplasm in which the shape and size of the nuclei are comparable and nuclear distribution is uniform. These features were recently shown to be essential for muscle function. In an attempt to elucidate mechanisms regulating the position and shape of myonuclei, we analyzed the function of the two KASH proteins that are uniquely present in the *Drosophila* genome, MSP-300 and Klarsicht, both expressed in striated muscles. We demonstrated that both KASH proteins cooperate to construct a unique ring composed of MSP-300 protein that surrounds and attached to the nuclear envelope. The MSP-300 nuclear ring structure recruits and associates with a network of polarized astral microtubules that enables the dynamic movement and uniform spacing between the nuclei in each muscle fiber.

### Introduction

The shape of the nucleus and its positioning within the cytoplasm have been implicated as important factors in controlling chromosomal architecture and transcriptional regulation.<sup>1</sup> Striated fly muscles exhibit extremely ordered cytoplasm, in which the myonuclei are evenly spaced along the entire muscle fiber, distributed at the fiber periphery, close to the sarcolemma and separated from the acto-myosin compartment.<sup>2</sup> This cellular architecture is disrupted in various human myopathies,<sup>3,4</sup> including Emery Dreifuss muscular dystrophy (EDMD)<sup>5</sup> and arthrogryposis (in which embryonic movement is arrested)<sup>6</sup> suggesting a link

between nuclear positioning and muscle function. Importantly, recent evidence in the model organism, *Drosophila*, demonstrated a direct link between myonuclei positioning and proper muscle function, emphasizing the physiological significance of correct myonuclear positioning in proper movement of the organism.<sup>7–9</sup>

Striated muscle fibers are multinucleated cells of variable size, morphology and physiology (e.g., fast or slow muscles),<sup>10–12</sup> but in each of the distinct muscle types, the nuclei, as well as other cytoplasmic organelles are evenly distributed along the entire cytoplasm, suggesting a mechanism capable of sensing muscle dimensions, which is coupled to the cellular machinery capable of moving and/or anchoring organelles within the cell. Surprisingly, information regarding such mechanisms is extremely limited, possibly due to the inability to follow the dynamics of organelle positioning within contracting muscles in live organisms.

The involvement of KASH-SUN proteins in the regulation of nuclear positioning has been described in various cell types.<sup>13–17</sup> SUNs are inner nuclear membrane proteins, with their SUN domain located at their C-terminal end. This domain is inserted into the inner nuclear membrane and was recently shown to form trimers within the inner nuclear membrane.<sup>18,19</sup> The N-terminus of SUN proteins associates with LaminA/C, which forms part of the nuclear matrix, associated with chromosomal organization. KASH proteins are nuclear envelope associated proteins, which bind to a variety of cytoskeletal elements through their variable N-terminal domains. Their

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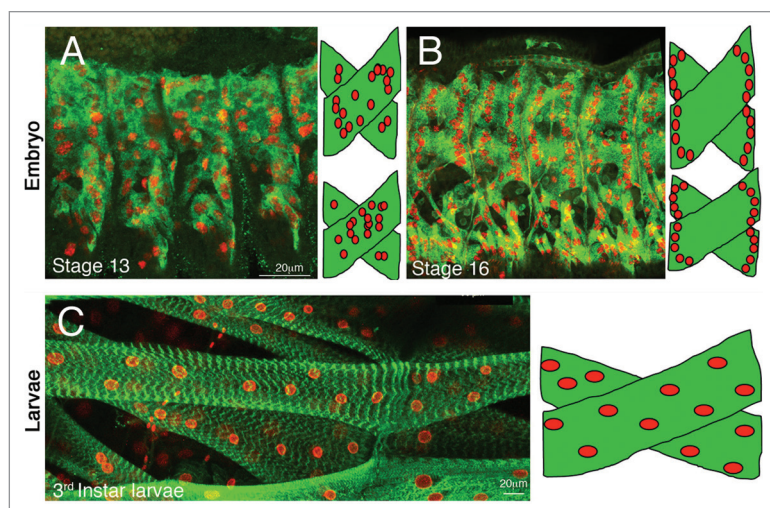
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**Figure 1.** Rearrangement of muscle nuclear organization during different stages of development. Wild type embryos stained for MHC (green) and MEF-2 (red) (A and B) at embryonic stage 13 (A) and stage 16 (B). Third instar larvae doubled stained with MHC (green) and Lamin (red) antibodies (C). The cartoon in each panel represents the relative distribution of the muscle nuclei. Originally published in Elhanany-Tamir H, et al. J Cell Biol 2012; 198:833-46. Reprinted with permission.

C-terminal KASH domain potentially forms trimers that directly associate with the SUN trimers at the perinuclear space.<sup>20</sup>

Distinct KASH proteins can associate with microtubules (MT) via interaction with MT motor proteins or with intermediate filaments and/or with the actin cytoskeleton.<sup>17</sup> Because KASH proteins are often co-expressed in more than one tissue, it is difficult to reveal their specific functional contribution to a given cell type. For example, Nesprin1 and Nesprin2 are both expressed in muscles as well as in neurons and their single or double knock down in mice induces a complex phenotype and early lethality (double KO)<sup>21</sup>; however, their specific contribution for the establishment of muscle fiber structure and function has been unclear.

To reveal the involvement of KASH proteins in the establishment of muscle fiber architecture as well as in muscle function, we analyzed the specific contribution of each KASH protein and the combination of both in *Drosophila* larval striated muscles.<sup>9</sup> The *Drosophila* genome contains only two KASH proteins, MSP-300 and Klarsicht (Klar); both are expressed in muscles. Below, I describe our most significant findings and present a molecular model for the specific activity of KASH proteins in promoting organelle positioning in muscles and explain

its physiological significance. This model will be discussed in view of recent studies suggesting novel molecular players in organelle positioning in muscles.

### Nuclear Positioning, but Not Number Changes During the Growth and Differentiation of Striated Muscles

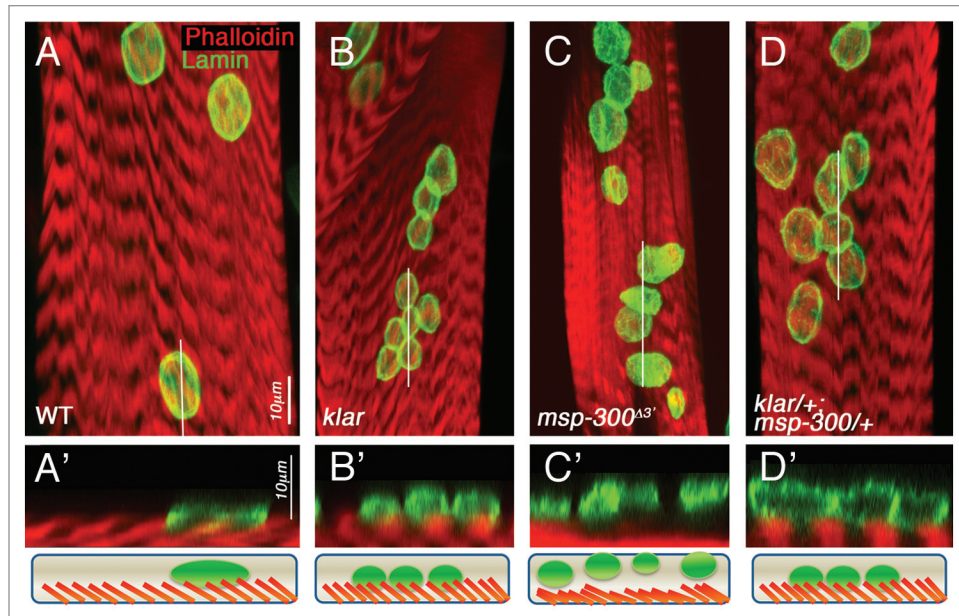
In *Drosophila*, multinucleated myotubes are formed by fusion of a fixed number of myoblasts to a muscle founder cell; the myotubes subsequently migrate and attach to a specific tendon cell.<sup>22</sup> Muscle striation takes place at a later developmental stage after the myotube has established its myotendinous junctions with tendon cells at both muscle ends.<sup>23</sup> Whereas the number of nuclei in each muscle type is fixed for a given muscle, the subcellular localization of the nuclei changes at each of these stages (see Fig. 1). Accordingly, the regulation of nuclear positioning differs at each developmental stage. This was shown by demonstrating the differential phenotypes of *klar* and *msh-300* homozygous mutant embryos. Whereas Klar is essential for myonuclear positioning in the non-striated myotubes, MSP-300 is dispensable. Conversely, in fully differentiated striated muscles, both MSP-300 and Klar are essential and cooperate in the induction

of nuclear positioning. Thus, myonuclear positioning is a dynamic process, which continuously accommodates nuclear distribution to the shape, dimensions and differentiation state of muscle fibers.

### Klar and MSP-300 Cooperate to Maintain Myonuclear Positioning in Striated Muscles

In *Drosophila*, the embryonic somatic muscles grow about 40-fold to accommodate the large size of the third instar larvae; however, the number of the myonuclei does not change. Both *klar* null mutants and *msh-300*<sup>Δ3</sup> mutants, in which the KASH and a large portion of the spectrin repeats are deleted, exhibit a muscle phenotype in which myonuclei are aggregated leading to significantly slower larval movement, consistent with the idea that myonuclear positioning is essential for muscle function (Fig. 2B and C). The autonomous requirement of each of these proteins in muscles was proven by a muscle-specific rescue (in the case of Klar) or by the muscle specific expression of MSP-300.

To differentiate between parallel vs. cooperative function of both Klar and MSP-300 proteins, the phenotype of double-heterozygous larvae carrying a single mutant allele for each of these genes was analyzed. Single heterozygous larvae for either *klar* or *msh-300* mutant alleles exhibit wild type muscles in which the nuclei are distributed in a wild type pattern and larval locomotion is normal (data not shown). However, double heterozygous *klar*<sup>+/+</sup>; *msh-300*<sup>+/+</sup> larvae exhibit a significant disruption of myonuclear positioning (Fig. 2D), thus they interact genetically. The genetic interaction between the two KASH proteins suggests functional cooperation between these proteins in promoting proper myonuclear positioning. Further evidence for such cooperation between Klar and MSP-300 was deduced from aberrant subcellular distribution of MSP-300 in *klar* mutant muscles. In wild type muscles, MSP-300 forms a specialized ring surrounding each nucleus and is connected to the Z-discs (Fig. 3A). In *klar*, or in *msh-300* mutant muscles, the MSP-300 nuclear ring dissociates from the nuclei but is still detectable (Fig. 3B and C), whereas in the double mutant *klar*; *msh-300* the

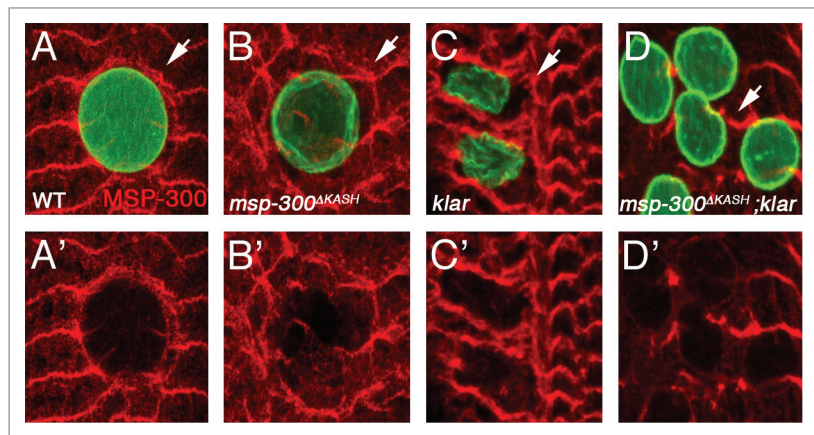


**Figure 2.** Nuclear aggregation and morphology depends on both Klar and MSP-300. Somatic muscles in third instar larvae, of wild type (A), *klar* (B), *msp-300* (C), double *klar/+;msp-300/+* (D) mutants, labeled with phalloidin (red) and anti Lamin (green). Note the aggregation of the nuclei and their aberrant morphology in the mutants. Orthogonal optical cross sections along the white line indicated in (A–D) are shown below each panel, demonstrating the tight association of the nuclei with the acto-myosin compartment in all the mutants except of the *msp-300* mutant (C). The schemes below each panel illustrate the distance between the nuclei and the acto-myosin compartment. Originally published in Elhanany-Tamir H, et al. J Cell Biol 2012; 198:833–46. Reprinted with permission.

MSP-300 nuclear ring completely disappears (Fig. 3D), consistent with the idea that the MSP-300 nuclear ring associates with the nuclear envelope via the KASH domain of both proteins. Significantly, both MSP-300 and Klar appear to participate in a single protein complex as indicated by co-immunoprecipitation experiments. Therefore, it appears that the MSP-300 nuclear ring is tethered by the cooperative activity of both MSP-300 and Klar KASH domains and represents a key structure essential for proper distribution of nuclei within muscle fibers.

### The MSP-300 Nuclear Ring Bridges Between Polarized Astral Microtubule Network Surrounding Each Nucleus

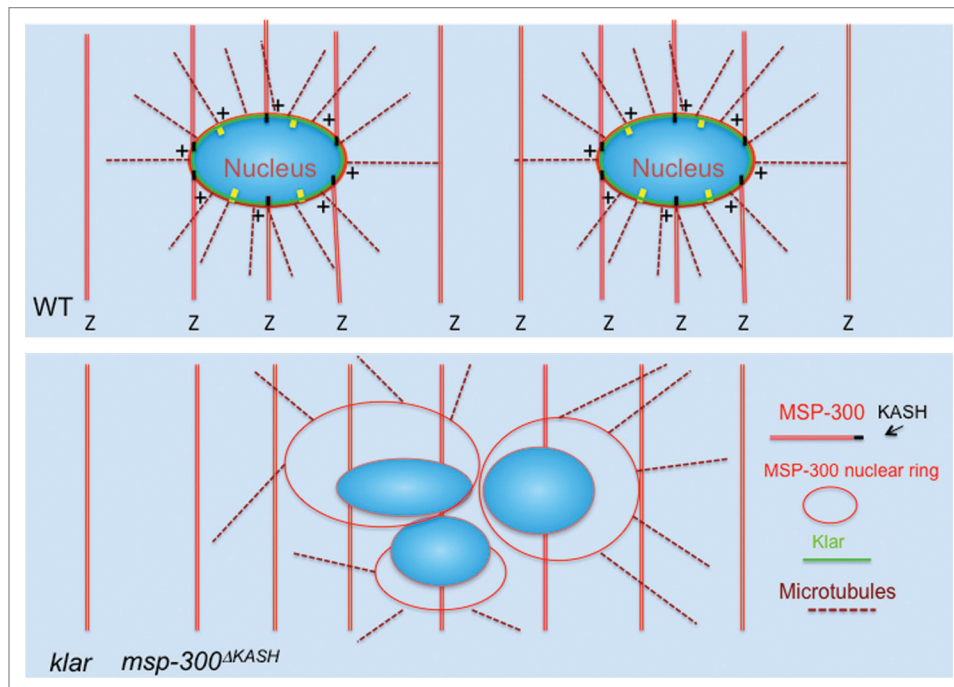
Subsequent analysis revealed the contribution of the MSP-300 nuclear ring to the attachment of a specialized network of polarized MT to the nuclear envelope. Microtubule organization is a dynamic process that is controlled in a spatial and temporal manner in every cell type. Developing muscles show a dynamic distribution of MT that changes at different



**Figure 3.** The association of the MSP-300 nuclear ring depends on both Klar and MSP-300. Larval muscles labeled with anti MSP-300 (red) and Lamin (green). (A and A') wild type muscles, (B and B') *msp-300<sup>ΔKASH</sup>*, (C and C') *klar* and (D and D') *msp-300<sup>ΔKASH</sup>;klar* double mutant muscles. The MSP-300 nuclear ring is indicated by arrow in all panels. Note the dissociation of the MSP-300 nuclear ring from the nuclear envelope in the mutants and its complete disruption in the double mutants. Originally published in Elhanany-Tamir H, et al. J Cell Biol 2012; 198:833–46. Reprinted with permission.

developmental stages. For example, prior to the development of sarcomeric architecture, the MT are arranged with their plus ends facing the ends of the muscles close to the myotendinous junction.<sup>24</sup> However, in fully striated larval muscles, the MT at

the surface are arranged in an astral organization with their plus ends facing each nucleus and in association with the MSP-300 nuclear ring<sup>9</sup> (Fig. 4, upper panel). It is assumed that the minus ends are distal to the nuclear envelope, although this has



**Figure 4.** The link between MSP-300 nuclear ring and the astral microtubules. Upper panel, wild type muscle with nuclei surrounded by Klar (green) and by MSP-300 nuclear ring (red), both associated with the astral microtubules (dashed brown lines). MSP-300 at the Z-bands (double red lines) associates with MSP-300 nuclear ring. The KASH domain of MSP-300 and Klar (black line) anchors the nuclei to MSP-300 nuclear ring and to the astral microtubules. Lower panel, in both *klar* and MSP-300<sup>KASH</sup> mutant muscles, the MSP-300 nuclear ring dissociates from the nuclei, as well as the astral microtubules. This leads to aggregation of the nuclei and abrogation of their normal size and shape.

not been tested directly. Importantly, in *klar* mutant or in *msp-300* lacking the KASH domain (*msp-300*<sup>KASH</sup>), the astral MT detach from the nuclear envelope but are still partially associated with the remnants of MSP-300 nuclear ring (Fig. 4, lower panel). These results suggested that the association of the astral MT with the nuclear envelope is mediated by a cooperative function of MSP-300 and Klar proteins containing the KASH domain together with the MSP-300 nuclear ring. Based on these results, we propose a model in which the astral MT together with the MSP-300 nuclear ring and Klar form a single unit that translocates the nuclei to induce their uniform spacing. Additional MT associated proteins, including the MT associated protein, Enscin (an ortholog of mammalian MAP7) and kinesin heavy chain are actively involved in this process.<sup>7</sup> Their association with the nuclear envelope might be mediated by binding to Klar, previously demonstrated to contain binding domains for both the plus and minus MT motor proteins.<sup>25</sup> In addition, based on the reported association between Nesprin-2 and Kinesin light chain, which

was demonstrated in keratinocytes<sup>26</sup> it is possible that MSP-300 (homologous to Nesprin) associates with the Kinesin light chain, as well. The dynamic nature of nuclear translocation within muscles is manifested by the involvement of both opposing MT motors in this process<sup>27</sup> but the mechanism whereby this translocation is temporally controlled is yet to be elucidated. A similar process of nuclear separation and their even distribution was described within the early syncytium *Drosophila* embryo.<sup>28</sup> In this process, the involvement of astral MT surrounding each nucleus and attached to actin-related structures was demonstrated. Whether these proteins participate in nuclear positioning within the large striated muscle cell is, as yet, unknown.

#### Anchoring the Nuclei to the Acto-Myosin Compartment Is a Unique Function of MSP-300

To enable proper order of the organelles in muscle fibers, which continuously contract, it is essential to physically anchor the organelles to existing structures within

the muscle cytoplasm. Our phenotypic analysis demonstrated that this role is unique to MSP-300 and is not shared with Klar and MT. Furthermore, the spacing and positioning of other organelles such as mitochondria and ER are also severely affected only in *msp-300* but not in *klar* mutant muscles. How MSP-300 mediates this activity is not clear. In contrast to Klar, which is uniquely detected only at the nuclear envelope, MSP-300 is also expressed along the Z-discs. Because both the nuclei and the mitochondria as well as ER structures are distributed on both sides of the Z-discs, we hypothesize that MSP-300 physically connects each organelle to the Z-discs, explaining the phenotype of *msp-300* mutant larvae and placing MSP-300 as a key protein in promoting positioning of all muscle organelles.

#### Concluding Remarks and Open Questions

Nuclear positioning is a dynamic process, which is tightly coupled to a given cell type, its state of differentiation, morphology and overall size. The results obtained

so far suggest that KASH proteins that intimately interact with the nuclear envelope continuously sense and respond to the dynamic changes in organization and polarization of the MT as well as the actin cytoskeleton. These interactions enable the nuclei and other organelles to change their position in response to growth and differentiation. The next challenge in this field will be to elucidate the dynamic properties that enable the KASH proteins to respond to the changing cytoplasmic environment within the muscles.

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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